

ECOGENOMIC ANALYSIS OF VERY DEEP SUBSURFACE ENVIRONMENTS AS ANALOGUES TO LIFE ON MARS

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RESEARCH OBJECTIVES

Terrestrial subsurface ecosystems have been proposed as analogues to life on other planets. Subsurface environments may provide habitable niches, protecting microorganisms from surface-related stress such as ultraviolet radiation, although at a cost. These environments are typically isolated from standard terrestrial energy economies (i.e., those revolving around solar energy): temperatures in the deep subsurface can reach 60°C. As such, microbes inhabiting these deep-subsurface ecosystems must adapt to such harsh conditions and be capable of obtaining energy by other means. By studying such isolated ecosystems, we hope to better understand the thresholds of life and determine the feasibility of life, past and present, on other planets in our solar system.

APPROACH

We have obtained fracture water samples from 2–3 km below ground level from multiple gold mines of the Witwatersrand Basin in South Africa. These water samples have been dated by isotopic methods as being 14 Kyr to 20 Myr old, with an energy system that appears to be sulfate- and hydrogen-based. To identify the microbial populations in the fracture water, we used a standard clone-library approach and a novel high-density microarray approach. Metagenomic analyses of extracted DNA were carried out at the DOE Joint Genome Institute and at Berkeley Lab to investigate the physiological capabilities of the dominant organism(s).

ACCOMPLISHMENTS

A deep-branching clade of nearly identical *Desulfotomaculum*-like, 16S rDNA sequences (>99% homology) was identified as the dominant microorganism in the planktonic phase of the deepest (2–3 km depth), most saline fracture water. The closest cultured relative is *Desulfotomaculum kuznetsovii* (90% similarity). Further sequences detected by clone library and high-density-array analysis included relatively abundant Firmicutes (*Bacillus*) and less abundant Alpha-, Beta-, Gamma and Delta-proteobacteria, *Cyanobacteria*, *Chloroflexi*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Spirochaetes*, *Verrucomicrobia* and *Planctomycetes*. For metagenome analyses, 2.3 Mb of DNA was assembled into 53 scaffolds, and 2,140 likely protein coding genes were identified. Preliminary analyses indicate that the dominant *Desulfotomaculum*-like organism (DLO) represents a new family of organisms and is almost

certainly a SO₄ reducer, based on its genomic content. The gene repertoire used for SO₄ reduction is largely the same described for other sulfate-reducing bacteria (SRB), including both delta-proteobacteria and the archaeon *Archeoglobus fulgidis*. Although it is a gram-positive organism, it shares a surprising number of its closest homologs with a diverse set of SRB, including *Desulfovibrio vulgaris*, *D. alaskensis* G20, *D. psychrophila*, and *A. fulgidis*. A number of close homologs were also observed to related organisms such as the S-reducing *Geobacter* and *Desulfuromonas* spp., and the SO₄ oxidizing *Chlorobium tepidum*. This dominant DLO most likely has flagellar motility controlled by a relatively small set of methyl-accepting chemotaxis proteins.

SIGNIFICANCE OF FINDINGS

Clone libraries and high-density microarrays allowed us to profile both the dominant and nondominant fraction of the microbial community in this sulfate/hydrogen-driven subsurface ecosystem. Through metagenome sequencing, we are able to gain insight into the growth strategy

of the dominant *Desulfotomaculum*-like organism, which appears capable of sulfate reduction linked to either hydrogen or formate oxidation, in addition to chemotactic motility. This data will enable a better understanding of adaptation and survival of microorganisms in isolated deep-subsurface systems.

RELATED PUBLICATIONS

Brockman, F., D. Moser, T. Gihring, D. Culley, E. Brodie, G. Andersen, T.C. Hazen, P. Richardson, L. Pratt, and T.C. Onstott, Inferred bioenergetics of an uncultured bacterium common in fracture fluids of South African deep mines. NASA Astrobiology Institute (submitted abstract), Biennial Meeting, Boulder, Colorado, 2005.

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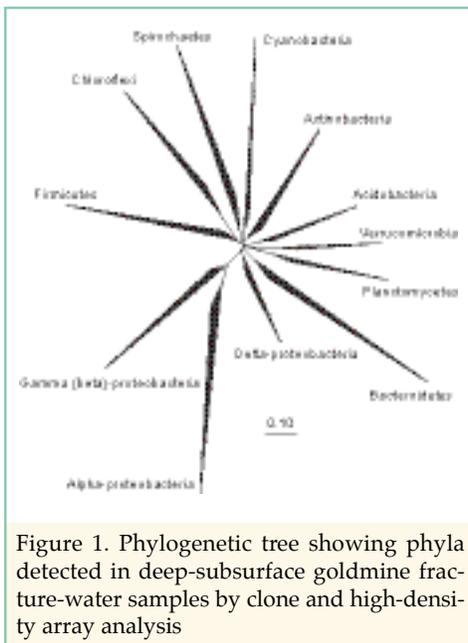


Figure 1. Phylogenetic tree showing phyla detected in deep-subsurface goldmine fracture-water samples by clone and high-density array analysis

